

PATENT COOPERATION TREATY

NO. 1120 D. 2/10

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 22 SEP 2005

WIPO PCT

See Form PCT/IPEA/416

Applicant's or agent's file reference
P65914PC00

FOR FURTHER ACTION

International application No.
PCT/EP2004/006805

International filing date (day/month/year)
18.06.2004

Priority date (day/month/year)
20.06.2003

International Patent Classification (IPC) or national classification and IPC
C12N9/50, C07K14/81

Applicant
NESTEC S.A. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 12 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
 - a. ☒ sent to the applicant and to the International Bureau a total of 3 sheets, as follows:
 - ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
 - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in Item 4 of Box No. I and the Supplemental Box.
 - b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
 - ☒ Box No. I Basis of the opinion
 - ☐ Box No. II Priority
 - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☒ Box No. IV Lack of unity of invention
 - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - ☐ Box No. VI Certain documents cited
 - ☐ Box No. VII Certain defects in the international application
 - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand

14.01.2005

Date of completion of this report

26.09.2005

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Page, M

Telephone No. +49 89 2399-7322



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**International application No.
PCT/EP2004/006805**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-43 as originally filed

Sequence listings part of the description, Pages

1-23 as originally filed

Claims, Numbers

1-23 received on 29.08.2005 with letter of 26.08.2005

Drawings, Figures

1-22 as originally filed

- ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**International application No.
PCT/EP2004/006805**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
- ☐ the entire international application,
 - ☒ claims Nos. 9-12 (completely) and 17-23 (partially)
because:
 - ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):
 - ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - ☒ no international search report has been established for the said claims Nos. 9-12 (completely) and 17-23 (partially)
 - ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
 - the written form ☐ has not been furnished
 - ☐ does not comply with the standard
 - the computer readable form ☐ has not been furnished
 - ☐ does not comply with the standard
 - ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.
 - ☐ See separate sheet for further details

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**International application No.
PCT/EP2004/006805**Box No. IV Lack of unity of invention**

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
- ☐ restricted the claims.
 - ☒ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. 1-8, 13-16 (completely) and 17-23 (partially).

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-8, 13-16 (completely), 17-23 (partially)
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	1-8, 13-16 (completely), 17-23 (partially)
Industrial applicability (IA)	Yes: Claims	1-8, 13-16 (completely), 17-23 (partially)
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY

International application No.
PCT/EP2004/006805

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)

International application No.

PCT/EP2004/006805

The application concerns the provision of eight separate polypeptides from the coffee plant. The application ascribes protease or protease inhibitor activity to these proteins, although it is not clear how the Applicant arrives at this assumption, as neither chemical tests nor functional sequence alignments are provided. The intention of the application is to influence coffee flavour through manipulation of the genes encoding these proteins.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

- III.1 The Applicant has elected not to pay additional search fees for Invention III as defined below under IV.2 (claims 9-12, pertaining to a polypeptide corresponding to SEQ ID NO. 6 or 8). As a result, no opinion regarding novelty or inventive step can be rendered for this subject matter.

Re Item IV

Lack of Unity of Invention

- IV.1 The application seeks protection for eight separate polypeptides and their corresponding polynucleotides. Two of the polypeptides have been nominated as cysteine proteinases (SEQ ID NOs. 2 and 16), four as cysteine proteinase inhibitors (SEQ ID NO. 4, 10, 12 and 16) and two are apparently related aspartic endoproteinases (SEQ ID NOs. 6 and 8). There is no technical feature disclosed in the application that unites these polypeptides in a manner that elevates them as a group over the prior art. The group of polypeptides does not have any discernable defining structural features that would distinguish them from sequences already known in the art. In fact certain sequences are more closely related to sequences already known in the art than to each other (see e.g. Uniprot accession number Q9ARH0, 73% identical to SEQ ID NO. 10 over its entire length).
- IV.2 Although the claims specify that the various sequences have defined functions as recited above, the application as filed does not indicate the basis for these assumed activities either through biochemical tests or by clear demonstration of the presence of known

INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)

International application No.

PCT/EP2004/006805

function-defining sequence motifs. Although sequence alignments are given for SEQ ID NOs. 2 and 16 with a number of known cysteine proteases (Fig. 2), there is no indication in the application that the polypeptides described by these SEQ ID NOs. also possess this activity. It appears, for example, for the given polypeptides that the degree of sequence identity can actually mislead the skilled person in ascribing function on this basis; the closest prior art for SEQ ID NO. 10 is Q9ARHO, a sequence sharing 73% sequence identity. The Applicant identifies SEQ ID NO. 10 as being a cysteine proteinase inhibitor, whereas Q9ARHO is known to be a cysteine protease. It is clear, therefore, that assigning function based on about 70% sequence identity is not reliable for the given polypeptides. For these reasons, the function of the sequences cannot be used as a technical feature in establishing unity of invention.

IV.3 The only basis under which unity of invention can therefore be assessed is the structural similarity (sequence identity) between the various sequences. Sequence alignment between the 6 claimed sequences yields the following % sequence identities:

SEQ ID NO.	16	14	12	10	4	2
2	28	3	4	6	7	100
4	10	9	26	30	100	
10	5	14	37	100		
12	12	22	100			
14	6	100				
16	100					

IV.4 The requirements of Rule 13.1 PCT are therefore not met and each of the claimed sequences can thus be regarded as a distinct invention. In view of the effort involved in searching and examining the claimed subject matter, however, the application is divided up into the following four distinct inventions, as defined by the claims:

Invention I (claims 1-4 (completely), 17-23 (partially))

Polynucleotide encoding SEQ ID NO. 2 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

22. 02. 2005 10.00
BIA MÜNCHEN V110 T49 0323994400

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

NK, 1/20 D. 9/16

International application No.

PCT/EP2004/006805

Invention II (claims 5-8 (completely), 17-23 (partially))

Polynucleotide encoding SEQ ID NO. 4, 10, 12 or 14 or polypeptides at least 80% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Invention III (claims 9-12 (completely), 17-23 (partially))

Polynucleotide encoding SEQ ID NO. 6 or 8 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Invention IV (claims 13-16 (completely), 17-23 (partially))

Polynucleotide encoding SEQ ID NO. 16 or polypeptides at least 70% identical thereto, vectors, transformed cells and a method for modulating coffee flavour.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 Reference is made to the following documents:

D1: DATABASE EMBL [Online] 29 April 1994 (1994-04-29), NONG, V. ET AL.: XP002269560 retrieved from EBI accession no. EMBL Database accession no. Z32795

D2: DATABASE UNIPROT [Online] 1 March 2002 (2002-03-01); YAMADA, K. ET AL.: XP002269561 retrieved from EBI accession no. UNIPROT Database accession no. Q8VYS0

D3: MARRACCINI PIERRE ET AL: "Molecular cloning of the complete 11S seed storage protein gene of Coffea arabica and promoter analysis in transgenic tobacco plants" PLANT PHYSIOLOGY AND BIOCHEMISTRY, GAUTHIER-VILLARS, PARIS, FR, vol. 37, no. 4, April 1999 (1999-04), pages 273-282, XP002197483 ISSN: 0981-9428

D4: LEROY T ET AL: "GENETICALLY MODIFIED COFFEE PLANTS EXPRESSING THE BACILLUS THURINGIENSIS CRY1AC GENE FOR RESISTANCE TO LEAF MINER" PLANT CELL REPORTS, SPRINGER VERLAG, DE, vol. 19, no. 4, 2000, pages 382-389, XP001002322 ISSN: 0721-7714

D5: WO 02/04617 A (KOCHHAR SUNIL ;NESTLE SA (CH); BUCHELI PETER (FR);

INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)

International application No.

PCT/EP2004/006805

- LALOI MARYSE (F) 17 January 2002 (2002-01-17)
- D6: WO 02/42327 A (KOCHHAR SUNIL ;NESTLE SA (CH); HANSEN CARL ERIK (CH); JUILLERAT MA) 30 May 2002 (2002-05-30)
- D7: LING J-Q ET AL: "Cloning of two cysteine proteinase genes, CysP1 and CysP2, from soybean cotyledons by cDNA representational difference analysis" BIOCHIMICA ET BIOPHYSICA ACTA , GENE STRUCTURE AND EXPRESSION, ELSEVIER, AMSTERDAM, NL, vol. 1627, no. 2-3, 19 June 2003 (2003-06-19), pages 129-139, XP004431612 ISSN: 0167-4781
- D8: DATABASE USPTO Proteins [Online] 14 February 2001 (2001-02-14), "Sequence 74 from patent US 6103514." XP002310749 retrieved from EBI accession no. USPOP:AAE48221 Database accession no. AAE48221
- D9: DATABASE Geneseq [Online] 17 October 2000 (2000-10-17), "Arabidopsis thaliana protein fragment SEQ ID NO: 36701." XP002310750 retrieved from EBI accession no. GSN:AAG30665 Database accession no. AAG30665

V.2 Novelty - Art.33(1) and (2) PCT:

2.1 Invention I (claims 1-4 (completely), 17-23 (partially))

The subject matter of **claims 1-4** appears to be novel in light of the available prior art. Only partial sequences show significant levels of sequence identity with SEQ ID NO. 2 and no sequences could be found having 70% or more identity to SEQ ID NO 2 over its entire length. The sequences disclosed at the time of filing with the highest degree of identity to those of the application are as follows:

SEQ ID NO.	Length	Prior Art	Length	% Sequence Identity	Overlap
1	1543 nt	D1: Z32795	1441 nt	75.5%	702 nt
2	397 aa	D2: Q8VYS0	367 aa	70.1%	380 aa
2	397 aa	D9: AAG30665	277 aa	73.8%	287 aa

2.2 Invention II (claims 5-8 (completely), 17-23 (partially))

The subject matter of **claims 5-8** is novel in light of D7. Sequences 80% or more

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2004/006805

identical to SEQ ID NO. 10 are not described in the prior art.

SEQ ID NO.	Length	Prior Art	% Sequence Identity	Overlap (parent:prior art)
4	139 aa	ø	-	-
10	98 aa	Q8VX72	72.2%	1-90:1-90
10	98 aa	D7: Q9ARH0	73.2%	1-98:1-98
10	98 aa	Q9M4Q4	68.4%	1-98:1-101
12	124 aa	ø	-	-
14	119 aa	ø	-	-

ø - no relevant sequence found

2.3 Invention IV (claims 13-16 (completely), 17-23 (partially))

The subject matter of **claims 13-16** appears to be novel in light of the cited prior arts. D7 discloses a polypeptide which is 70.6% identical to SEQ ID NO. 16 over its entire length. The claims are directed to sequences sharing 80% or sequence identity however. The sequences disclosed at the time of filing with the highest degree of identity to those of the application are as follows:

SEQ ID NO.	Length	Prior Art	% Sequence Identity	Overlap (parent:prior art)
16	359 aa	D7: Q7X750	70.6%	1-359:1-362
16	359 aa	CYSP_VIGMU	69.8%	1-359:1-362
16	359 aa	D8: AAE48221	69.8%	1-359:1-362

2.4 None of the anticipated sequences are disclosed as being used in modulating coffee flavour. Accordingly, **claims 1-8 and 23** appear to be novel in light of the cited prior art.

V.3 Inventive Step - Art.33(1) and (3) PCT:

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2004/006805

- 3.1 Although the cited prior art does not disclose a solution to the technical problem of modulating coffee flavour precursor levels, the application is not considered to have demonstrated inventive step. The Applicant states on page 3 of the description that differences exist in the levels and amounts of the major storage proteins in green coffee and that small differences exist between the storage proteins of immature and mature coffee beans, which have different flavour qualities, the link between the two is not conclusively made, though evidence exists which suggests that altering amino acid concentrations in foods may affect flavour, for example in coffee, through the Maillard reaction (Fay and Brevard 2005: 24:487-507; mentioned by the Applicant). However, it is not clear that the claimed polypeptide can directly or indirectly affect either the levels of the said storage proteins or amino acids and thus the flavour of the resulting beans. Because the activity of the claimed proteins has not been established by the Applicant, the application is not considered to disclose a method of altering the levels of proteins, peptides and amino acids that might result in a flavour change.
- 3.2 In order for inventive step to be acknowledged, the Examiner is obliged to satisfy him or herself that the objective technical problem has indeed been solved by the application in suit. As this cannot be said to be the case, inventive step is not acknowledged.

Re Item VII**Certain defects in the international observation**

- 1 The application lacks support from the description. The eight polypeptide sequences disclosed have been designated as cysteine proteinases (SEQ ID NOs. 2 and 16), cysteine proteinase inhibitors (SEQ ID NO. 4, 10, 12 and 16) and two apparently related aspartic endoproteinases (SEQ ID NOs. 6 and 8). However, the application fails to indicate how the function of these proteins were determined in the absence of any biochemical evidence to this end. The application is therefore not considered to fulfill the requirements of Article 5 PCT.
- 2 The claimed invention is not sufficiently disclosed for the skilled person to be able to

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/EP2004/006805

modulate coffee flavour precursor levels. The Applicant speculates that introducing the genes provided which encode the eight polypeptides into coffee plants will influence the flavour of the resulting coffee. There is no evidence provided in the application that makes this assumption credible, in fact the description rather indicates that this is uncharted territory.

The Applicant speculates on page 3 that the proteases and protease inhibitors of the application will alter the amino acid and small peptide composition of the beans, but there are apparently no reports directly linking specific levels or ratios of amino acids and high or low flavour qualities. Also no association between these storage protein differences and flavour quality has been noted, and currently no clear evidence exists linking any differences seen for the coffee storage proteins, or other major green bean proteins, and the flavour qualities of coffee.

Against this backdrop, the Applicant provides a number of proteases and protease inhibitors and claims a method for altering coffee flavour without indicating (a) whether these enzymes actually do play a role in flavour development, (b) how the flavour is ultimately altered by these proteins and © what promoters to use for the alteration of flavour in terms of tissue specificity, stage specificity and expression levels.

The most that the Applicant can be said to have done is to compare the expression levels of the various proteins between over time and between *C. arabica* and *C. canephora*. However, no correlation is made between expression levels and particular flavour qualities and there is no indication that the differences in flavour over time or between species are caused or influenced by these proteins. Regarding the promoter, the application cites a paper by Leroy *et al.* from 2000 in which coffee is transfected with a gene under the control of the CaMV promoter, but there do not appear to be any native coffee promoters used in the art.

There is further no worked example teaching the skilled reader how the claimed invention can best be carried out (Rule 5.1(a)(v) PCT). There is clearly a chasm of information between the disclosure of the application and the proposed invention that the skilled person would not be in a position to span without considerable experimentation and inventive energy. The description states on page 5 that "an object of the present invention is to improve the flavour quality of coffee." The application fails to teach the skilled person how to do this and therefore fails to fulfil the requirements of Article 5 PCT.

SUGGESTED REVISED CLAIMS:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide
5 having cysteine proteinase activity, wherein the amino acid sequence of the polypeptide
and the amino acid sequence of SEQ ID No. 2 have at least 70%, preferably at least 80%,
sequence identity based on the ClustalW alignment method; or the complement of the
nucleotide sequence, wherein the complement contains the same number of nucleotides as
10 the nucleotide sequence, and the complement and the nucleotide sequence are 100%
complementary.
2. The polynucleotide of Claim 1, wherein the amino acid sequence of the polypeptide and
the amino acid sequence of SEQ ID No. 2 have at least 85%, preferably at least 90%,
15 optionally at least 95%, sequence identity based on the ClustalW alignment method.
3. The polynucleotide of Claim 1, wherein the nucleotide sequence comprises the nucleotide
sequence of SEQ ID No. 1.
4. The polynucleotide of Claim 1, wherein the polypeptide comprises the amino acid
20 sequence of SEQ ID No. 2.
5. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide
having cysteine proteinase inhibitor activity, wherein the amino acid sequence of the
25 polypeptide and the amino acid sequence selected from the group consisting of SEQ ID
Nos. 4, 10, 12 and 14 have at least 80%, sequence identity based on the ClustalW
alignment method; or the complement of the nucleotide sequence, wherein the
complement contains the same number of nucleotides as the nucleotide sequence, and the
complement and the nucleotide sequence are 100% complementary.
- 30 6. The polynucleotide of Claim 5, wherein the amino acid sequence of the polypeptide and
the amino acid sequence selected from the group consisting of SEQ ID Nos. 4, 10, 12 and
14 have at least 85%, preferably at least 90%, optionally at least 95%, sequence identity
based on the ClustalW alignment method.

7. The polynucleotide of Claim 5, wherein the nucleotide sequence comprises the nucleotide sequence selected from the group consisting of SEQ ID Nos. 3, 9, 11 and 13.
8. The polynucleotide of Claim 5, wherein the polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID Nos. 4, 10, 12 and 14.
9. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide having aspartic endoproteinase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence selected from SEQ ID No. 6 or 8, preferably SEQ ID No. 8, have at least 75%, preferably at least 80%, sequence identity based on the ClustalW alignment method, or the complement of the nucleotide sequence, wherein the complement contains the same number of nucleotides as the nucleotide sequence, and the complement and the nucleotide sequence are 100% complementary.
10. The polynucleotide of Claim 9, wherein the amino acid sequence of the polypeptide and the amino acid sequence selected from SEQ ID No. 6 or 8, preferably SEQ ID No. 8, have at least 85%, preferably at least 90%, optionally at least 95%, sequence identity based on the ClustalW alignment method.
11. The polynucleotide of Claim 9, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID No. 5 or 7, preferably SEQ ID No. 7.
12. The polynucleotide of Claim 9, wherein the polypeptide comprises the amino acid sequence of SEQ ID No. 6 or 8, preferably SEQ ID No. 8.
13. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide having cysteine proteinase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID No. 16 have at least 80% sequence identity based on the ClustalW alignment method; or the complement of the nucleotide sequence, wherein the complement contains the same number of nucleotides as the nucleotide sequence, and the complement and the nucleotide sequence are 100% complementary.

14. The polynucleotide of Claim 13, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID No. 16 have at least 85%, preferably at least 90%, optionally at least 95%, sequence identity based on the ClustalW alignment method.
- 5 15. The polynucleotide of Claim 13, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID No. 15.
16. The polynucleotide of Claim 13, wherein the polypeptide comprises the amino acid sequence of SEQ ID No. 16.
- 10 17. A vector comprising the polynucleotide of any one of Claims 1 to 16.
18. A non-native recombinant DNA construct comprising the polynucleotide of any one of Claims 1 to 16 operably linked to a regulatory sequence.
- 15 19. A method for transforming a cell comprising transforming a cell with the polynucleotide of any one of Claims 1 to 16.
20. A cell comprising the non-native recombinant DNA construct of Claim 18.
- 20 21. The cell of Claim 20, which is a prokaryotic cell, an eukaryotic cell or a plant cell, preferably a coffee cell.
22. A transgenic plant comprising the cell of Claim 20 or 21.
- 25 23. A method for modulating coffee flavour precursor levels in green coffee grains, the method comprising introducing into the coffee plant the non-native recombinant DNA construct of Claim 18.